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A Paternally Derived Inverted Duplication of 7q With Evidence of a Telomeric Deletion

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We report on a de novo constitutional rearrangement involving the long arm of chromosome 7 in a second trimester fetus with the karyotype of 46,XX, inv dup del (7)(pter-q36::q36-q21.2:) pat. Both a large duplication (q21.2-q36) and a small deletion (within q36) were confirmed by FISH studies. DNA analysis on the family showed that the abnormal chromosome was derived from a single paternal homolog. A mechanism is proposed in light of this finding. The phenotype at autopsy was consistent with reported cases of similar duplications in chromosome 7 in that hydrocephalus, a depressed nasal bridge, low set ears, microretrognathia and a short neck were present. Am. J. Med Genet. 68: 76–81, 1997

KEY WORDS: inverted; duplication; deletion; 7q

INTRODUCTION

De novo duplications (direct or inverted) are rare forms of structural rearrangements involving a single chromosome. Although infrequently reported in the long arm (q) of chromosome 7, inverted duplications are relatively common in the short arm (p) of chromosome 8, where a characteristic phenotype has been described. Increasing evidence suggests that in most cases of inv dup 8p a small telomeric deletion also exists [Dill et al., 1987; Mitchell et al., 1994; Minelli et al., 1993; Barber et al., 1994; Guo et al., 1995] and meiotic mechanisms have been proposed to account for the derivation of some of these chromosomes [Mitchell et al., 1994; Guo et al., 1995].

A similar type of complex rearrangement resulting in both a large duplication (q21.2-q36) and a small deletion (within q36) is reported here in the long arm of one chromosome 7. The phenotype is compared to cases of partial duplication of 7q found in the literature [reviewed by Forabosco et al., 1988], most being the result of unbalanced translocations. In our case, fluorescence in situ hybridization (FISH) confirmed the composition of the abnormal chromosome, the small deletion in band q36 and the presence of a telomeric sequence on qter. DNA analysis indicated the abnormal 7 originated from a single paternal homolog suggesting that the abnormality could have arisen either in mitosis in the spermatogonia, in spermatogenesis or during early embryogenesis.

CLINICAL REPORT

A healthy 26-year-old Caucasian primigravid woman of German/Polish descent was admitted at 20 weeks gestation for premature rupture of membranes 2 weeks prior to admission. Until this time the pregnancy had been uncomplicated, with a normal maternal serum triple marker screen. The patient had used two applications of topical Retin-A during the first 4 weeks of pregnancy; she denied exposure to other drugs, chemicals, infections, or possible teratogenic agents. The father was 30 years old, Caucasian, of Irish/Latvian descent, and in good health. The family history was notable for the patient's maternal grandmother who had two miscarriages and a set of stillborn twins in addition to 13 healthy children. The family history was otherwise unremarkable for this non-consanguineous couple.

Obstetrical sonography demonstrated fetal nuchal thickening and moderately dilated lateral cerebral ventricles as well as marked oligohydramnios. The couple was counseled about the likelihood of pulmonary hypoplasia and the possible causes of the fetal abnormalities. The options of chorionic villus sampling for cytogenetic analysis, continuation of the pregnancy, and pregnancy termination were discussed and the couple elected to terminate. Labor was induced by prostaglandin suppositories and the patient delivered a 310 g female fetus at $20^{5}/_{7}$ weeks. A skin biopsy was obtained after delivery for chromosome studies.

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Autopsy showed minor anomalies including a flat nose with slightly anteverted nostrils, lowset, posteriorly rotated flat ears, and microretrognathia (Fig. 1a,b). There was excess skin at the back of the neck with mild webbing. The palate was intact but high-arched. The fetus had a sacral dimple, with no evidence of underlying skeletal abnormality, and clinodactyly and camptodactyly with ulnar deviation. The shoulders and hips were held in internal rotation with webbing of the joints in the axillary and inguinal regions. The knees exhibited genu valgus and there was talipes equinus of both feet with prominent heels. The lateral ventricles were enlarged, while the third and fourth ventricles were of normal size and the cerebral aqueduct was patent.

Cytogenetic Analysis

Chromosome studies were carried out on fetal skin and on peripheral lymphocytes from the parents using standard G banding methods. FISH was performed according to the method of Pinkel et al. [1986, 1988]. The chromosome 7 library, pBS 7, was a gift from J.W. Gray and was labeled with biotin-14-dATP by nick translation (BRL) and 200 ng were preannealed with 26 μg/ml human placental DNA for 90 minutes at 37°C. The probe and slides were denatured separately with 70% formamide for 5 minutes in 2XSSC, pH 7, at 75°C. The Elastin Williams syndrome chromosome region (WSCR) probe identifies 7q11.23 and contains as a control probe D7S427, which maps to 7q36 (Oncor, Gaithersburg, MD). The map location of this cosmid, D7S427, was reported in 1993 at the first international workshop on human chromosome 7 [Grzeschik et al., 1994]. This locus is in the proterminal region adjacent to the highly conserved human-specific terminal repeat array present at the telomeres of all human chromosomes [Hing et al., 1993]. The probe was used, according to the manufacturer's instructions, to identify the q subtelomeric region of chromosome 7. An all-telomere probe was combined with a chromosome-specific α-satellite probe (Oncor) in order to determine if a telomere was present within the q arm or at the q terminus of the abnormal chromosome 7. All hybridizations were carried out at 37°C and probes were detected with avidin conjugated to fluorescein or anti-digoxigenin antibody with a detection kit from Oncor. Chromosomes were counter-

Fig. 1. Fetus at autopsy at 20⅚ weeks showing (**a**) minor facial anomalies including a depressed nasal bridge, low set ears, and microretrognathia. Internal rotation of the shoulders and hips, bilateral genu valgus, and talipes equinus with prominent heels can be seen and (**b**) excess nuchal skin with mild webbing and clinodactyly and camptodactyly of both hands.

stained with propidium iodide in Vectashield antifade (Vector Laboratories, Burlingame, CA) and viewed using a Zeiss epifluorescence microscope equipped with a broad band-pass FITC filter cube. Approximately 20 cells were studied in each hybridization experiment.

DNA ANALYSIS

DNA analysis was carried out on cultured lymphocytes from the parents, fixed for cytogenetic studies in Carnoy's fixative (3 parts methanol:1 part acetic acid), and stored at 4°C for several months [Shibo et al., 1995], and on cultured skin fibroblasts from the fetus. Samples were analyzed using three highly polymorphic dinucleotide repeat markers (Genethon), D7S486, D7S550, and D7S530 mapped to 7q with heterozygosity values of 0.81, 0.83, and 0.78, respectively. DNA was isolated using a Puregene DNA Isolation Kit (Gentra). Lymphocytes were pelleted, washed with 70% ethanol, and recentrifuged prior to isolation. PCR was carried out in 20 µl volumes using 0.2 µM primers, the forward primer being labeled with a blue fluorescent dye (FAM), 0.2 mM each dNTP, 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and 1 U Taq polymerase (Perkin-Elmer). The PCR was performed in a Perkin-Elmer 9600 Thermocycler. After heating to 94°C for 3 minutes, the reaction was cycled according to the following program: 2 cycles of 20 seconds at 94°C, 20 seconds at 62°C, 20 seconds at 72°C; 2 cycles of 20 seconds at 94°C, 20 seconds at 61°C, 20 seconds at 72°C; etc. to 10 cycles of 20 seconds at 94°C, 20 seconds at 52°C, 20 seconds at 72°C. One microliter of the reaction was analyzed on a Model 373 DNA Sequencer (Perkin-Elmer Applied Biosystems Division) using Genescan software (PE ABD). Each sample was run with an internal lane standard (Genescan-500) of a known concentration. Results were imported into a Genotyper (PE ABD) for allele determination.

RESULTS

Cytogenetic analysis of fetal skin cells showed a karyotype of 46,XX, inv dup del (7)(pter-q36::q36-q21.2:) in all 20 cells studied. G-banded homologs of chromosomes 7 and an ideogram are shown (Fig. 2). Parental karyotypes were normal.

FISH with a chromosome 7 library confirmed the composition of the abnormal chromosome and no additional hybridization signals on other chromosomes were seen (Fig. 3a). In order to further define the chromosome, the 7q sub-telomeric cosmid probe, D7S427, was used in a hybridization experiment. This probe gave a clear signal at the tip of the long arm of the normal chromosome 7 but gave no suggestion of a signal at either the telomere or the interstitial region of the abnormal 7, thought to contain duplicated copies of band q36 (Fig. 3b). In each case simultaneous hybridization with a chromosome 7-specific WSCR probe correctly identified the chromosome. A probe for sequences common to all human telomeres was used to confirm the presence of this sequence at the tip of the long arm of the abnormal chromosome 7 (Fig. 3c). In this experiment the conditions of the hybridization were maximized for the telomere probe causing the α-satellite probe to loose some specificity. However, chromosomes 7 could still be easily identified.

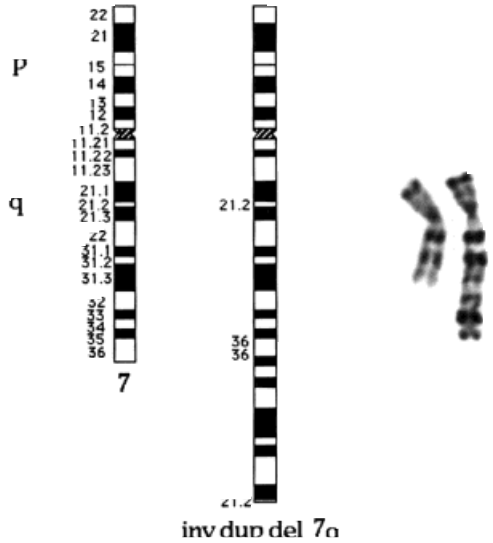


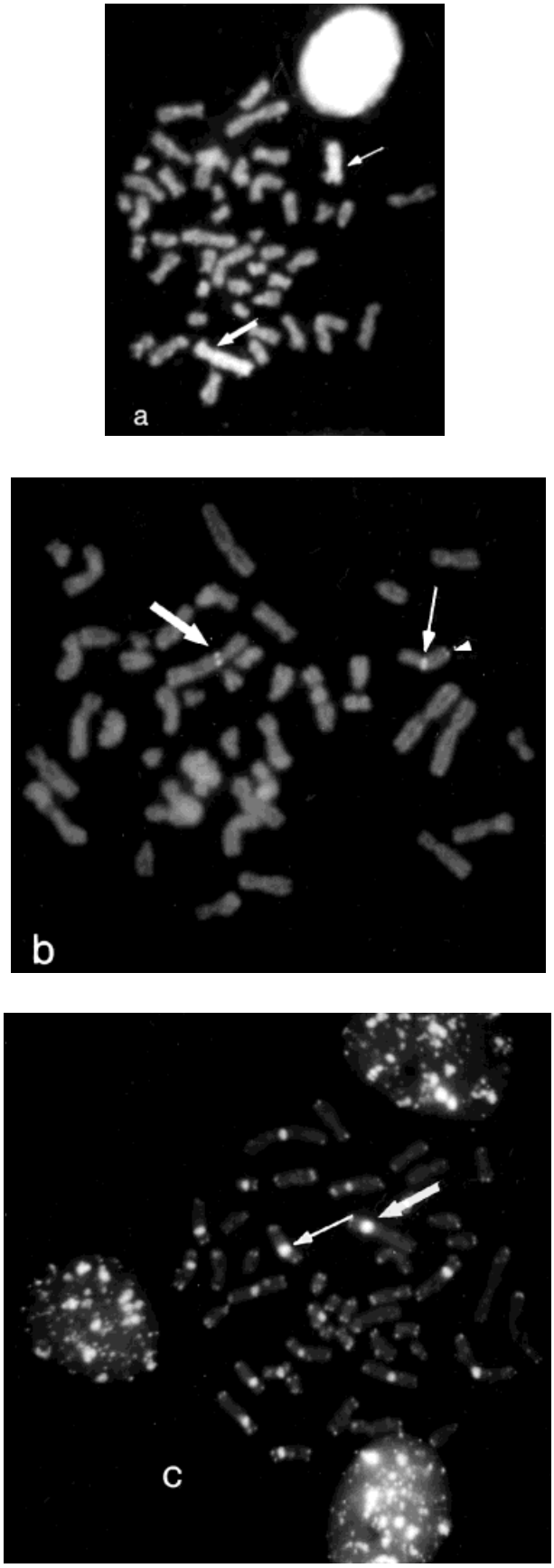
Fig. 2. G-banded chromosomes 7 from the fetus (right) and an ideogram showing the inv dup del 7q.

DNA studies on this family using three highly polymorphic markers in chromosome 7q indicated that the fetus had received one copy of chromosome 7 from the mother and that the abnormal chromosome contained a duplication in 7q entirely from a single paternal chromosome. Of these three markers D7S486 is closest to the centromere, D7S530 is more distal and D7S550 is closest to the telomere [NIH/CEPH Collaborative Mapping Group, 1992]. D7S486 and D7S530 are 9 cM apart and D7S550 is 49 cM distal to D7S530 [Weissenbach, 1992]. All three markers showed an increase in signal intensity of the paternally inherited allele as compared to the maternal allele. For example, for both D7S486 and D7S550, the fetus clearly inherited two copies of one paternal chromosome rather than a single allele from each paternal chromosome (Fig. 4). Unrelated samples with the same base pair difference between alleles were used as controls for signal intensity and area comparison (data not shown). The area ratio between the two alleles in the fetus is 1.4 to 2.35 times the value measured in the control group alleles.

DISCUSSION

Both traditional and molecular cytogenetic studies have been used to describe a de novo constitutional rearrangement in the long arm of chromosome 7. G banding suggested an inverted duplication involving bands q21.2-q36. The chromosome was shown to be entirely of chromosome 7 origin. Part of band q36 is apparently deleted from the abnormal chromosome as shown by the absence of hybridization with the telomeric probe, D7S427. A telomere, however, is present at the q terminal end of this chromosome, as shown with an all-telomere probe, presumably the result of de novo synthesis [Wilkie et al., 1990].

De novo inverted duplications in the long arm of chromosome 7 are apparently quite rare, with only two previously reported cases [Kardon et al., 1980; Haslam



and Norman, 1992], one of which is very similar to our case in both phenotype and karyotype [Haslam and Norman, 1992]. A review of the literature on partial trisomy 7q showed that most cases are the result of unbalanced translocations inherited from balanced reciprocal translocation carriers and a small number of cases result from a parental paracentric inversion. Findings at autopsy in our case were consistent with the more than 40 previously reported cases [Forabosco et al., 1988; Haslam and Norman, 1992; Bartsch et al., 1990; Stratton et al., 1993] in that hydrocephalus, depressed nasal bridge with upturned nares, low set ears, microretrognathia and a short neck were present. Unlike most previous reports, the fetus reported here showed abnormal positioning of shoulders, elbows, and hips accompanied by some webbing of the neck and joints. The webbing indicates the fetus had abnormal positioning for considerably longer than would have been expected from the date of rupture of membranes. Abnormalities involving the extremities have not been a prominent feature of partial trisomy for 7q. Nonetheless, joint contractions of the extremities have been previously reported in dup(7)(q33-qter) [Bartsch et al., 1990] and in dup(7)(q21-qter) [Stumpner, 1989]. Talipes equinovarus has been reported in a case involving a de novo inv dup (7)(q22-q36.1) [Haslam and Norman, 1992] and in one involving a familial derived dup(7)(q21-qter) [Forabosco et al., 1988]. In the later case there was documented presence of amniotic fluid at delivery, and in the former case antenatal sonography had been performed without mention of any abnormality in amniotic fluid volume. Flexion deformities in the fingers have been infrequently described in the phenotype; however, this has been reported in a case of dup(7)(q32-qter) [Couzin et al., 1986] and in one involving dup(7)(q21-qter) [Begleiter et al., 1995].

Monosomy for a portion of band 7q36 was also detected in our analysis. Deletions of 7q36-qter have been associated with holoprosencephaly [Lurie et al., 1990], and it is possible that this band contains the locus responsible for this effect [Hatziioannou et al., 1991]. However, holoprosencephaly was not present in our case and mapping data concerning the exact sub-band location of the probe used in this analysis (D7S427) was not available.

In our case DNA studies showed that the abnormal chromosome 7 was entirely paternal in origin, being derived from a single paternal chromosome, and this suggests a mitotic derivation of the abnormal chromosome. One mechanism that may have led to this unusual chromosome 7, which contains both an inverted duplication (q21.2-q36) and a deletion (q36), would involve two events. First, a sister chromatid fusion within band q36 during meiosis or mitosis would account for loss of

Fig. 3. FISH showing chromosomes 7 from the fetus with **a**) the chromosome 7 library, **b**) the sub-telomeric probe mapping in 7q36 (note hybridization to band q36 in the normal 7 only, indicated with an arrow head), and **c**) an all-telomere probe in combination with a chromosome 7 α-satellite probe (note a telomere on the inv dup del 7q). Dark arrows indicate the inv dup del 7 and pale arrows the normal 7 in each photograph.

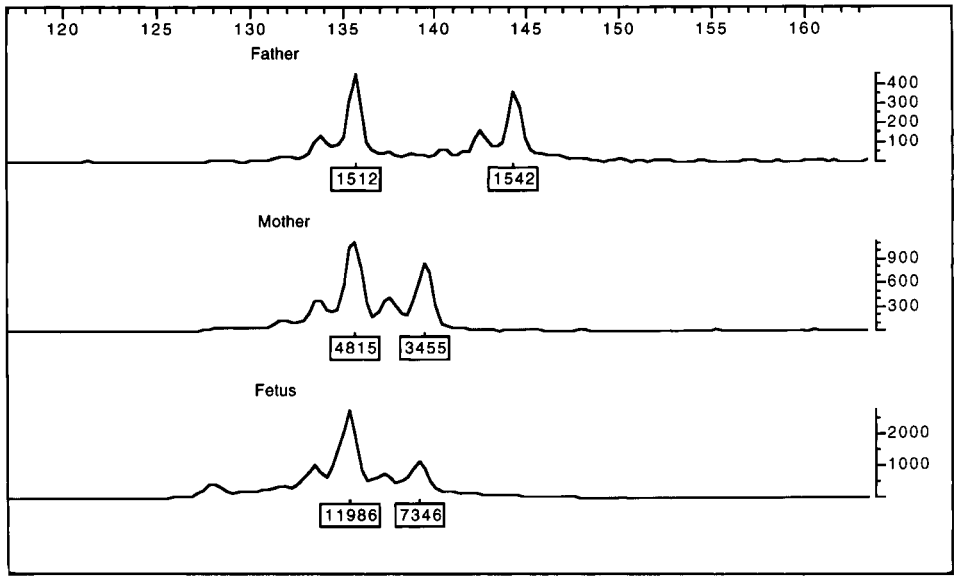


Fig. 4. Electropherogram of the dinucleotide repeat marker D7S486. Forward primers were labeled with a phosporamidite and resolved on a model 373 DNA Sequencer (Applied Biosystems). Data were collected and analyzed with Genescan 672 software (Applied Biosystems) which calculates fragment size in reference to an internal lane standard and quantitates the amount of fluorescence of unknown PCR products from which allele specific information is interpreted. The data are imported into the Genotyper program (Applied Biosystems) for automated allele calling. Genotyper differentiates the alleles from the "stutter" bands using a filter algorithm designed for dinucleotide microsatellites. Data are presented in chromatogram form; the x-axis shows the time (number of base pairs) and the y-axis shows the peak heights (amount of fluorescence). The boxed numbers show the peak area. Alleles are labeled with the peak area.

material from band q36 and produce a dicentric chromosome (pter-cen-q36::q36-cen-pter) showing molecular identity for the entire duplicated region. This dicentric chromosome, being unstable, could then break in band q21.2 at the following anaphase (pter-cen-q36::q36-q21.2:). Healing of the broken end with telomerase [Wilkie et al., 1990; Morin, 1991; Lamb et al., 1993] would produce the stable monocentric chromosome reported here. The first event, occurring within one replicated chromosome, could have taken place in the mitotically dividing spermatogonia, during paternal meiosis or in an early mitotic division of the embryo, but probably not at a later point since telomerase is present in germline cells but thought to be absent or inactive in most human somatic cells [Cooke and Smith, 1986; Blackburn and Greider, 1995]. Somatic origin of a similar chromosome has been recently proposed for a case of mosaicism of a dup del 8p [Priest, 1995], with the assumption that the event occurred at a time when telomerase was still functioning. In our case, mosaicism for a normal cell line was not detected in the fetus.

Alternative mechanisms proposed to explain similar inv dup del 8p [Mitchell et al., 1994; Guo et al., 1995] involve end-to-end fusion or a U-type exchange within a paracentric inversion loop. These mechanisms are limited to meiosis I and, although they could produce similar chromosomes to the one described here, our data limits the initial event to a sister chromatid fusion. If a sequence is determined to predispose to crossing over errors between homologs or sister chromatids in chromosome 8p, it would be useful to look for a similar sequence in 7q.

It is interesting to note that complex de novo chromosome rearrangements are more likely to be paternal than maternal in origin [Batista et al., 1993], presumably as a result of environmental exposure, or possibly a consequence of the great number of divisions of the spematogenic process. In the case reported here, however, no exposure to known clastogenic agents was reported by the patient or her husband. In order to decipher the derivation of de novo chromosomal duplications in the future it will be important for cytogenetics laboratories to utilize FISH techniques to check these unusual chromosomes for small terminal deletions and to use chromosomal or DNA polymorphisms to determine the parental origin. Only in this way can we arrive at appropriate mechanisms for these unusual errors in human reproduction.

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